Steroid metabolome analysis reveals that prostate cancer has potent 5α-reductase, 3α- and 17β-hydroxysteroid dehydrogenase activities, but lacks 17-hydroxylase/17,20-lyase

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GP-30-07

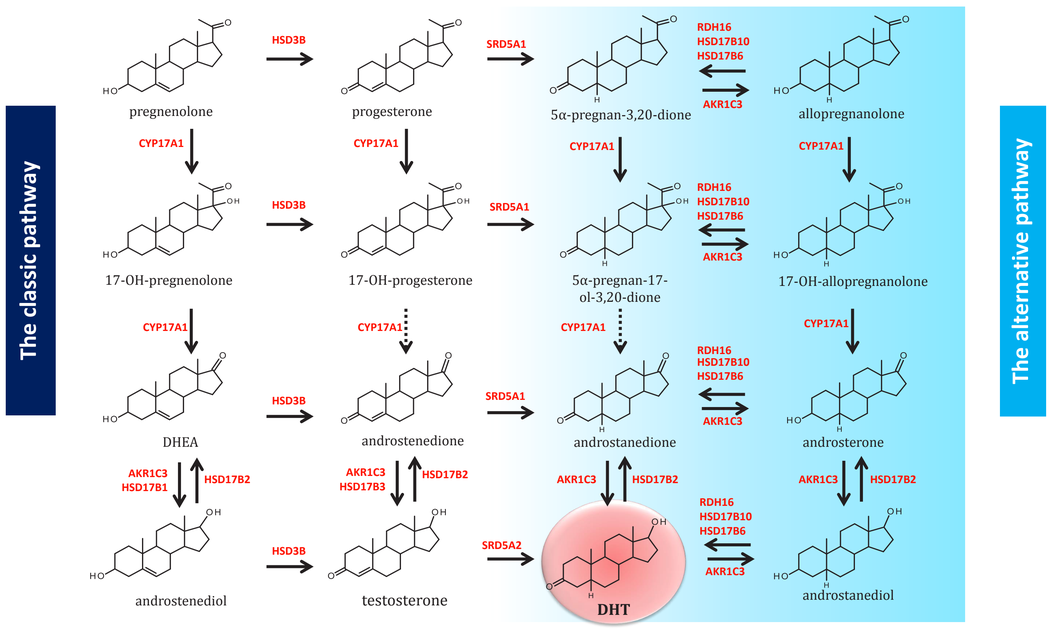
Introduction

Prostate cancer (PC) is dependent on androgen receptor (AR) activation by its canonical ligands testosterone and 5α-dihydrotestosterone (DHT). Intratumoural androgens persisting after castration give rise to castration-resistant PC (CRPC). These intraprostatic androgen levels are hypothesized to result from either adrenal androgen conversion or intratumoural *de novo* DHT synthesis through the classic or alternative pathways. Quantifying the steroid fluxes responsible for CRPC development can help optimize current endocrine treatment strategies.

Methods

Five common PC cell lines were incubated with 1 µM of 16 steroid intermediates of the classic and alternative androgen synthesis pathways. A PC steroid metabolome was constructed through measurement of steroid metabolite concentrations with liquid chromatography/tandem mass spectrometry (LC-MS/MS). Expression of steroidogenic enzymes and AR-responsive genes was estimated by quantitative PCR.

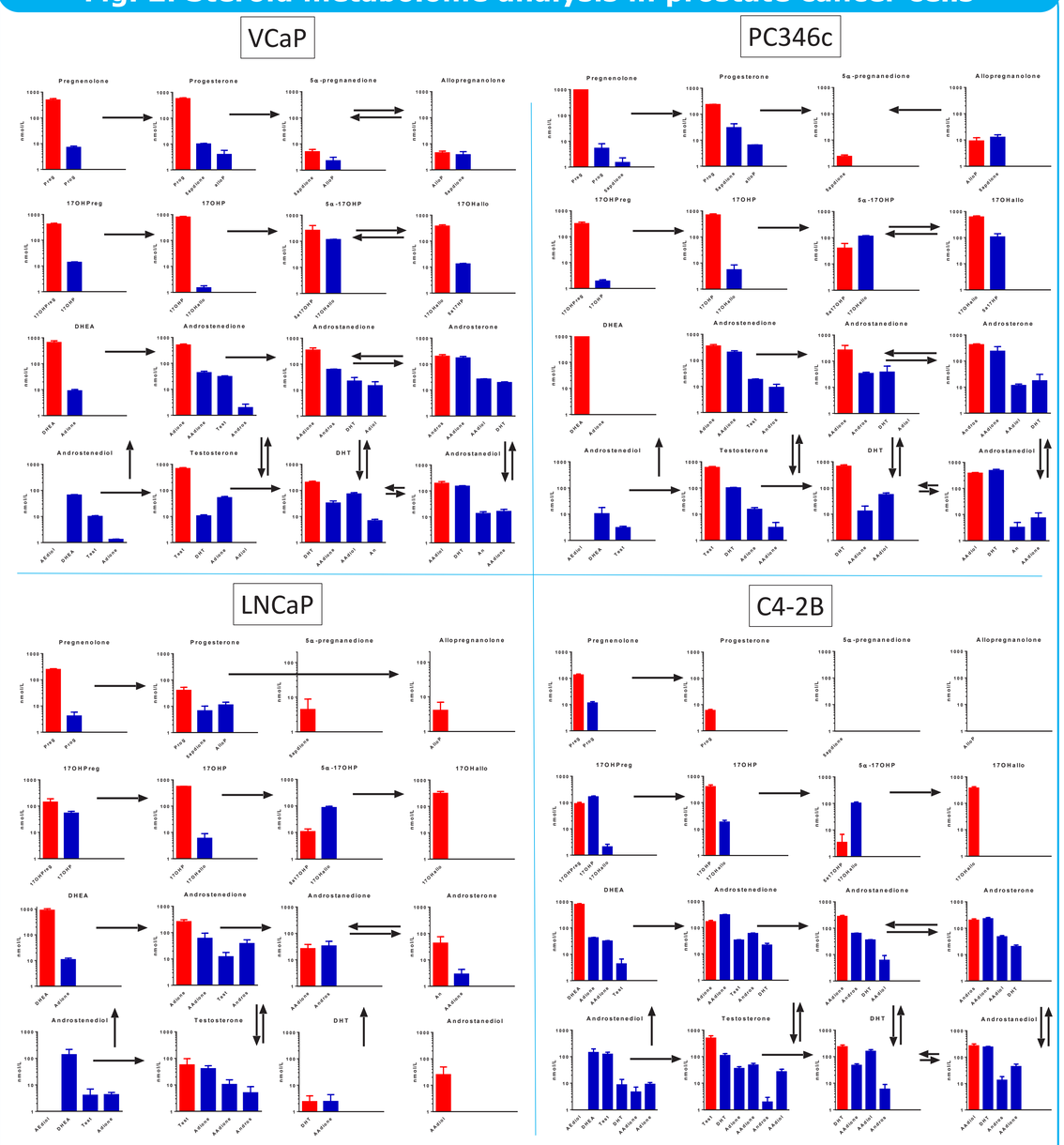
Fig. 1: Steroidogenic pathways



Common steroid precursor cholesterol is converted into pregnenolone by side-chain cleavage. Multiple steroidogenic enzymes convert pregnenolone into the active androgen dihydrotestosterone (DHT) through either the classic pathway (white) or the alternative pathway (light blue).

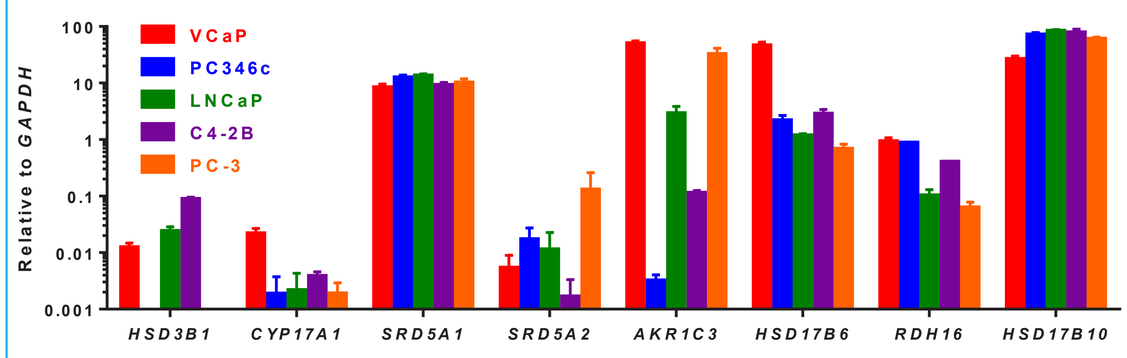
Results

Fig. 2: Steroid metabolome analysis in prostate cancer cells



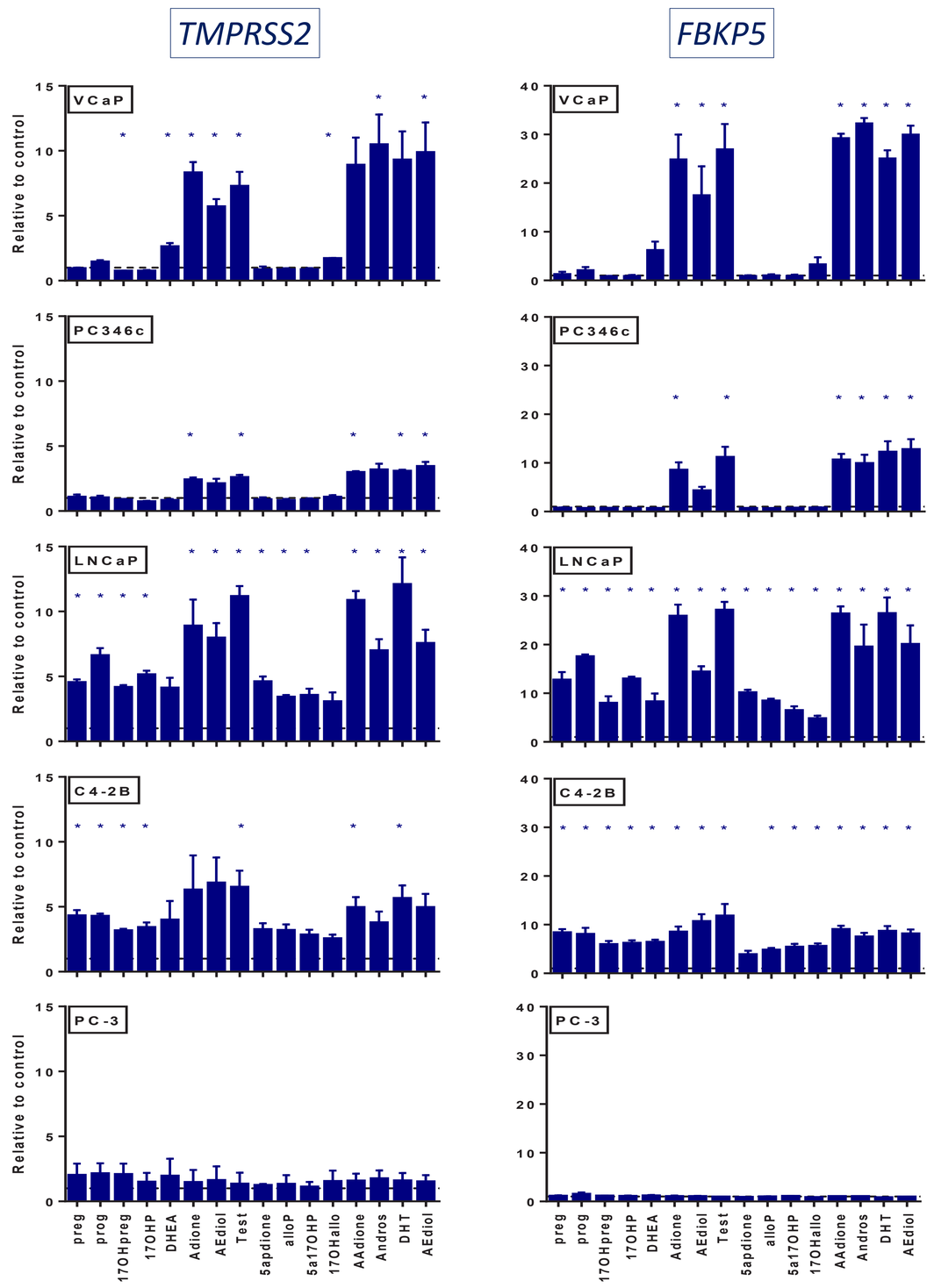
Four prostate cancer cell lines were incubated with 1 µM of steroid hormones. Supernatant levels of incubated steroids (red) and their metabolites (blue) were measured through LC-MS/MS.
No endogenous pregnenolone production or CYP17 activity was detected.
Flux into the alternative pathway occurred from both progesterone as well as 17OH—progesterone.
Downstream of CYP17 there was potent conversion of androgen (metabolites) in all cell lines tested.
C4-2B, a bone-metastasizing/castration-resistant clone of LNCaP, showed the highest steroidogenic potential.

Fig. 3: Basal levels of steroidogenic enzymes



mRNA expression in prostate cancer cell lines, measured by QPCR.
Enzymes for *de novo* steroidogenesis were expressed at low to absent levels, whereas SRD5As and HSD17Bs were differentially expressed at higher levels. This reflects the steroidogenic activities observed in the metabolome analysis (Fig. 2).

Fig. 4: AR activation by steroid hormones



mRNA expression of AR-responsive genes *TMPRSS2* and *FKBP5* in prostate cancer cell lines after 24H incubation with 16 steroid hormones (Fig. 1). Values are measured by QPCR and calculated relative to vehicle.
Steroids upstream of *CYP17A1* only influenced expression in LNCaP and C4-2B cells, that harbour the AR T877A mutation. Steroids downstream of *CYP17A1* trigger AR transactivation in all AR-positive cell lines.
This reflects the steroidogenic activities observed in the metabolome analysis (Fig. 2).

Conclusions

- First quantitative steroid metabolome of PC cells.
- No evidence supporting intratumoural *de novo* steroid synthesis or CYP17 activity.
- Precursor C21 steroids divert towards the alternative pathway. The presence of these hormones might suggest a role for activity or further metabolism of alternative pathway steroids in PC evolution.
- Adrenal androgens can effectively be converted into DHT in PC.

Funding

MARIE CURIE ACTIONS
Royal College of Physicians

17 ECE
7-GP-30
Endocrine tumours
Johannes Hofland
DOI: 10.3252/pso.eu.17ece.2015
Poster presented at: ECE2015 16-20 May 2015 DUBLIN, IRELAND 17th EUROPEAN CONGRESS OF ENDOCRINOLOGY
PosterSessionOnline.com